

# Radiation Resistance and Loss of Crystal Violet Binding Activity in *Yersinia enterocolitica* Suspended in Raw Ground Pork Exposed to Gamma Radiation and Modified Atmosphere

Saumya Bhaduri, Shiowshuh Sheen, and Christopher H. Sommers

**Abstract:** Virulence of many foodborne pathogens is directly linked to genes carried on self-replicating extra-chromosomal elements, which can transfer genetic material, both vertically and horizontally, between bacteria of the same and different species. Pathogenic *Yersinia enterocolitica* harbors a 70-kb virulence plasmid (pYV) that encodes genes for low calcium response, crystal violet (CV) binding, Congo red uptake, autoagglutination (AA), hydrophobicity (HP), type III secretion channels, host immune suppression factors, and biofilm formation. Ionizing radiation and modified atmosphere packaging (MAP) are used to control foodborne pathogens and meat spoilage. In this study, the effect of gamma radiation and modified atmosphere (air, 100% N<sub>2</sub>, 75% N<sub>2</sub>: 25% CO<sub>2</sub>, 50% N<sub>2</sub>: 50% CO<sub>2</sub>, 25% N<sub>2</sub>: 75% CO<sub>2</sub>, 100% CO<sub>2</sub>) were examined by using the CV binding phenotype, for the presence or absence of pYV in *Y. enterocolitica*, suspended in raw ground pork. All *Y. enterocolitica* serovars used (O:3, O:8, and O5,27) were more sensitive to radiation as the CO<sub>2</sub> concentration increased above 50%. Crystal violet binding following a radiation dose of 1.0 kGy, which reduced the *Y. enterocolitica* serovars >5 log, was greatest in the presence of air (ca. 8%), but was not affected by N<sub>2</sub> or CO<sub>2</sub> concentration (ca. 5%). Following release from modified atmosphere after irradiation, the loss of CV binding rose from 5% to 8% immediately following irradiation to >30% after outgrowth at 25 °C for 24 h. These results, using *Y. enterocolitica* as a model system, indicate that the risk of foodborne illness could be affected by the loss of virulence factors when postprocess intervention technologies are used.

**Keywords:** gamma radiation, modified atmosphere, virulence plasmid, *Yersinia enterocolitica*

**Practical Application:** Provides gamma radiation D<sub>10</sub> data for inactivation data for *Y. enterocolitica* irradiated under modified atmosphere and information to risk assessors regarding the difference between pathogen presence versus actual virulence.

## Introduction

The presence of microbial pathogens in foods is a serious global problem, even in highly industrialized countries such as the United States. (Mead and others 1999; Scallan and others 2011). Despite the recent advances that have been made in food safety, the cost of foodborne illness is estimated to be approximately \$152 billion annually in the United States (Mead and others 1999).

The virulence of many foodborne pathogens such as Shiga-toxin producing and enterohemorrhagic *Escherichia coli*, *Salmonella* spp., and *Yersinia enterocolitica*, is directly linked to genes carried on plasmids, self-replicating extra-chromosomal elements, which can transfer genetic material, both vertically and horizontally, between bacteria of the same and different species (Butler and others 1987;

Johnson and Nolan 2009; Farshad and others 2012; Feng and others 2012). Environmental stresses such as chemicals, antibiotics, acidic environments, salt, ultraviolet light, and gamma radiation show differential effects on the maintenance of these large virulence plasmids in bacteria (Butler and others 1987; Cornelis and others 1998; Sommers and Bhaduri 2001). Understanding the role of plasmids, and specifically how to interrupt their transfer between microorganisms, is crucial to controlling the spread of human disease.

*Y. enterocolitica* causes an estimated 96000 cases of food-related illness in the United States annually (Mead and others 1999) and has been associated with many food types (Bhaduri 2014). The virulence of *Y. enterocolitica* is linked to the presence of 70-kb plasmid (pYV). A number of virulent phenotypes are expressed by pYV, including colony morphology/size (size = 1.13 mm), low-calcium response (Lcr, pin point colony, size = 0.36 mm), crystal violet (CV) binding (dark-violet colony), Congo red (CR)-uptake (red pin point colony, size = 0.36 mm), autoagglutination (AA = cells agglutinate), hydrophobicity (HP = forms clumps), type III secretion channels, host immune suppression, biofilm formation, and expression of a series of released proteins (Yops) (Cornelis and others 1998; Bhaduri 2014; Bhaduri and Smith 2013). Environmental stresses such as temperature, atmosphere,

MS 20131339 Submitted 9/20/2013, Accepted 1/19/2014. Author Bhaduri is with Molecular Characterization of Foodborne Pathogens Research Unit, U. S. Dept. of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA, 19038, USA. Authors Sheen and Sommers are with Food Safety and Intervention Technologies, U. S. Dept. of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA, 19038, USA. Direct inquiries to author Bhaduri (E-mail: saumya.bhaduri@ars.usda.gov). Mention of a brand or firm names does not constitute an endorsement by the U.S. Dept. of Agriculture over others of a similar nature not mentioned. USDA is an equal opportunity employer.

culture conditions, freezing, salt, acidic pH, and calcium concentration can affect virulence–plasmid associated phenotypes of *Y. enterocolitica*, *Y. pseudotuberculosis*, and *Y. pestis* such as Lcr, colony size, CV binding, CR–uptake, AA, and HP (Bhaduri and others 1988, 1993, 2011; Bhaduri 1995, 2005, 2006, 2011, 2014; Bhaduri and Sommers 2008; Bhaduri and Phillips 2011; Bhaduri and Smith 2012). *Y. enterocolitica* displays pYV-dependent formation of microcolonies in collagen gels, a system to mimic infection in living tissue when incubated at 37 °C. The expression of pYV-borne *Yersinia* genes can affect biofilm formation in other organisms *in vivo* and *in vitro* using gel matrices (Freund and others 2008; Atkinson and others 2011). The previously established DNA sequences of pYV in *Y. enterocolitica* and their associated phenotypes, coupled with well established methodologies to assess the presence and absence of pYV, make it an ideal model system to study effects of environmental stress and virulence.

Ionizing (gamma) radiation is a safe and effective technology, which is used on a global basis that uses no chemicals to inactivate pathogenic bacteria in food (FDA 1997). This technology inactivates foodborne pathogens by disrupting the genetic material, either killing them or rendering them unable to reproduce (Thayer and Boyd 1999). Modified atmosphere packaging (MAP) uses gas mixtures (for example, CO<sub>2</sub>, N<sub>2</sub>, O<sub>2</sub>) to slow the growth of microorganisms in food (Daniels, 1985). Ionizing radiation, in combination with modified atmosphere, especially atmospheres high in CO<sub>2</sub>, is better than each technology used individually to inactivate foodborne pathogens (Grant and Patterson 1991; Thayer and Boyd 1999; Kudra and others 2011).

Previous research from our laboratory demonstrated the effect of pYV on the radiation resistance of *Y. enterocolitica*, and the effect of ionizing radiation on maintenance of pYV in *Y. enterocolitica* (Sommers and Bhaduri 2001; Sommers and Novak 2002). The purpose of this study was to investigate the effect of ionizing radiation and modified atmosphere on the survival of *Y. enterocolitica*, as well as maintenance of pYV and assessment of CV binding activity.

## Materials and Methods

### Pork

Raw pork roast was purchased from a local market and ground through a 1/8-inch grinder plate. The ground pork (20% fat) was then aliquoted (100 g) into No. 400 Stomacher bags (Tekmar, Inc., Cincinnati, Ohio, U.S.A.) and vacuum-packed to 0.26 mm Hg using a Multi-Vac A300 Vacuum-Packager (Kansas City, Mo., U.S.A.). In order to eliminate contaminating microorganisms, the meat was then sterilized by irradiating it to a dose of 42 kGy (–30 °C). The meat was then stored at –70 °C until ready for use (Sommers and others 2002).

### *Y. enterocolitica*

Three different pYV-bearing strains, GER (serotype 0:3), ATCC 51871 (serotype 0:8), and PT18–1 (serotypes 0:5, 0:27), representing three serotypes of *Y. enterocolitica*, were used in this study (kindly provided by S. Weagant, Food and Drug Administration, Seattle, Wash., U.S.A.).

The isolates were propagated on Brain Heart Infusion Agar (BHA) (Difco Laboratories, Detroit, Mich., U.S.A.) at 27 °C and then maintained at 0 to 2 °C until ready for use. Species verification was performed using Gram Negative Identification cards using the Vitek Automicrobic System (bioMérieux, Inc., Hazelwood, Mo., U.S.A.). The presence of the pYV-borne *virF* gene,

as well as the chromosomal *ail* gene was verified by PCR (Sommers and others 2002). The presence of the pYV was also verified by plasmid DNA isolation and visualization via agarose gel electrophoresis (Sommers and others 2002).

### *Yersinia* propagation

*Y. enterocolitica* strains were cultured independently in 100 mL of Brain Heart Infusion Medium (BHI) (Difco Laboratories) using baffled 500-mL Erlenmeyer flasks at 27 °C (150 rpm) for 18 h. The bacteria were then sedimented by centrifugation (4 °C, 1725 × g) and resuspended as a cocktail in a tenfold reduced volume of the original culture in Butterfield's Phosphate Buffer (BPB) (Applied Research Inst., Newtown, Conn., U.S.A.). The individual strains were then mixed with 10 mL of bacteria 100-g sterile ground pork and mixed for 90 s in a Stomacher Mixer (Tekmar, Inc.).

### Packaging and modified atmosphere

The inoculated pork was then aliquoted (5 g) into thin a 1- to 2-mm layer in the bottom of a 100 × 15 mm Petri dish. The Petri dishes containing inoculated pork were then placed in gas impermeable Mil-B-131 (Bell Fibre Products, Columbus, Ga., U.S.A.), Type 1, 5.0 mil (0.127-mm thick) aluminum foil/polyethylene barrier pouches, which were then flushed twice with either air or modified atmosphere (100% N<sub>2</sub>, 75% N<sub>2</sub>: 25% CO<sub>2</sub>, 50% N<sub>2</sub>: 50% CO<sub>2</sub>, 25% N<sub>2</sub>: 75% CO<sub>2</sub>, 100% CO<sub>2</sub>) (Scotty Specialty Gases, Durham, N.C., U.S.A.) and then sealed using a Multivac A300 packager (Kansas City). Headspace was approximately 5:1. The samples were then stored overnight at 4 °C prior to irradiation. Overnight storage in MAP did not affect the viability of the *Y. enterocolitica*.

### Gamma irradiation

A Lockheed Georgia Company (Marietta, Ga., U.S.A.) self-contained <sup>137</sup>Cs irradiator was used for all exposures. The radiation source consisted of 23 individually sealed source pencils in an annular array. The 22.9 cm × 63.5 cm cylindrical sample chamber was located central to the array when placed in the operating position. Inoculated samples were placed vertically and centrally in the sample chamber, using a 4-mm-thick polypropylene bucket, to insure dose uniformity. The dose rate was 0.10 kGy/min.

The temperature during irradiation was maintained at the target by introduction of the gas phase from a liquid nitrogen source directly into the top of the sample chamber. Temperature was monitored by the use of 2 thermocouples, one placed centrally in the chamber and the other taped to the side of the sample bag. The absorbed dose was verified using 5-mm alanine pellets that were measured using a Bruker EMS 104 EPR Analyzer (Billerica, Mass., U.S.A.). The ionizing radiation doses were 0.2, 0.4, 0.6, 0.8, and 1.0 kGy.

### Head space gas analysis

Immediately before processing the samples for microbiological analysis a 0.5-mL sample was withdrawn from each sample bag and analyzed by gas chromatography using a Gow-Mac Series 580 Analyzer (Alltech Services, Inc., Deerfield, Ill., U.S.A.). The Analyzer was calibrated using commercially certified gas (Scotty I Nr. 9799, Scotty Specialty Gases, Inc., Durham, N.C., U.S.A.). All samples retained the appropriate gas mixture following storage and gamma irradiation.

After irradiation, the samples were then assayed for colony-forming units (CFU) by the standard pour plate method using BHA and 1/10 serial dilutions in BPB. Plates (10 per dilution)

were incubated at 37 °C for 1 d prior to scoring. CFU per plate, (30 to 300 per plate), were scored with a new Brunswick Scientific Biotran II colony counter for determination of  $D_{10}$ . Colonies were examined for the CV binding trait at each experimental condition using higher density plates.

PCR amplification of pYV-encoded and chromosomal DNA

Colonies which did not bind CV were randomly selected for PCR analysis for identity as *Y. enterocolitica* and absence of pYV (Sommers and Bhaduri 2001). Multiplex PCR using DNA primers to pYV-encoded regulatory gene *virF* and the chromosome-encoded *ail* gene (Bhaduri 2003) was used to verify the presence of pYV- DNA sequences and confirm *Y. enterocolitica* identity. Following PCR the amplified DNA fragments were separated via agarose gel electrophoresis, visualized with ethidium bromide and UV transillumination at 365 nm (Sommers and Bhaduri 2001).

D<sub>10</sub> values

The means of triplicate plate counts of the treated samples (*N*) were divided by the average control plate counts (*N*<sub>0</sub>) to give a survivor ratio (*N/N*<sub>0</sub>). The log<sub>10</sub>(*N/N*<sub>0</sub>) of the ratios were then used for determination of  $D_{10}$  values (Diehl 1995) and other statistical analyses.  $D_{10}$  values were determined by the reciprocal of the slopes following linear regression as determined by least squares analysis (15) (Sigma Plot, Version 5.0, Chicago, Ill., U.S.A.). The predictive equation for determination of log<sub>10</sub> survivor ratio's was performed using Sigma Plot Version 5.0. Analysis of Covariance (ANCOVA) and Analysis of variance (ANOVA) was performed using Statistical Analysis Software (SAS) Version 6.12 (SAS Inst., Cary, N.C., U.S.A.).

Crystal violet binding loss during post irradiation growth

Three samples per *Y. enterocolitica* isolate and gas mixture, including air, were set aside to assess pYV stability following exposure to gamma radiation (1.0 kGy). Packages were opened and allowed to incubate at ambient temperature (ca. 25 °C, 24 h). The crystal violet binding capability of the isolates was then determined as previously described. Figure 1 shows the presence and absence of pYV in *Y. enterocolitica* by CV binding technique (Bhaduri 2014).

Table 1–Radiation Resistance ( $D_{10}$ ) of *Y. enterocolitica* serovars in the presence of modified atmosphere.

Isolate/Serovar	Atmosphere	D-10 (SEM)	R <sup>2</sup>
GER/ O:3	Air	0.18 (0.02)	0.97
	100% N <sub>2</sub>	0.25 (0.02)	0.96
	75% N <sub>2</sub> : 25% CO <sub>2</sub>	0.24 (0.02)	0.98
	50% N <sub>2</sub> : 50% CO <sub>2</sub>	0.22 (0.01)*	0.97
	25% N <sub>2</sub> : 75% CO <sub>2</sub>	0.20 (0.02)*	0.98
51871/O:8	100% CO <sub>2</sub>	0.17 (0.02)*	0.96
	Air	0.17 (0.02)	0.95
	100% N <sub>2</sub>	0.23 (0.02)	0.96
	75% N <sub>2</sub> : 25% CO <sub>2</sub>	0.24 (0.02)	0.96
	50% N <sub>2</sub> : 50% CO <sub>2</sub>	0.20 (0.03)*	0.95
PT18–1/O:5	25% N <sub>2</sub> : 75% CO <sub>2</sub>	0.18(0.01)*	0.97
	100% CO <sub>2</sub>	0.15 (0.02)*	0.96
	Air	0.19 (0.01)	0.99
	100% N <sub>2</sub>	0.23 (0.01)	0.98
	75% N <sub>2</sub> : 25% CO <sub>2</sub>	0.23 (0.02)	0.95
Combined data	50% N <sub>2</sub> : 50% CO <sub>2</sub>	0.20 (0.01)*	0.97
	25% N <sub>2</sub> : 75% CO <sub>2</sub>	0.18 (0.01)*	0.98
	100% CO <sub>2</sub>	0.16 (0.02)*	0.95
	Air	0.18 (0.01)	0.99
	100% N <sub>2</sub>	0.24 (0.01)	0.99
	75% N <sub>2</sub> : 25% CO <sub>2</sub>	0.24 (0.01)	0.99
	50% N <sub>2</sub> : 50% CO <sub>2</sub>	0.21 (0.01)*	0.99
	25% N <sub>2</sub> : 75% CO <sub>2</sub>	0.19 (0.01)*	0.99
	100% CO <sub>2</sub>	0.16(0.01)*	0.99

The radiation  $D_{10}$  experiments were conducted 3 times for each isolate and modified atmosphere. Standard error of the mean is shown in parenthesis. The mean  $D_{10}$  for the 3 serovars is shown as combined data. Asterisks designate a statistical difference from the 100% N<sub>2</sub> atmosphere as determined by ANCOVA (*P* < 0.05).

Results and Discussion

Pork was chosen to suspend the *Y. enterocolitica* as the microorganism is a frequent contaminant of raw pork products and it represents a model system to examine the effect of food safety and preservation technologies on pYV maintenance and ultimately pathogen virulence (Sommers and Bhaduri 2001; Bhaduri 2014). The radiation resistance of *Y. enterocolitica*, suspended in raw ground pork, is shown in Table 1. The  $D_{10}$  values were consistent with those obtained in other studies (Grant and Patteron 1991; Kamat and others 1997; Sommers and Bhaduri 2001; Sommers and Novak 2002). Radiation sensitivity of the 3 *Y. enterocolitica* serovars was greatest in the presence of air, which is consistent with the

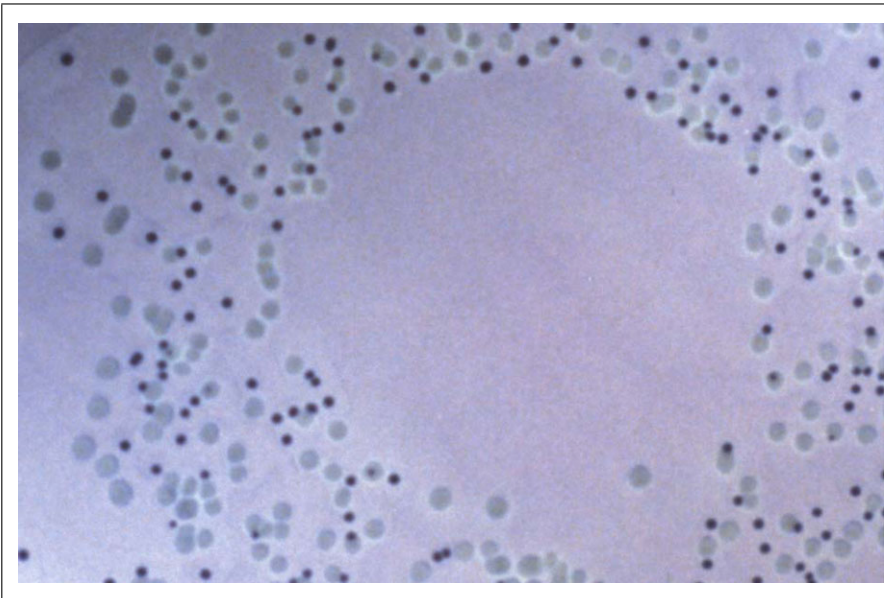


Figure 1–Detection of pYV strain in a mix culture of pYV-positive and isogenic pYV-less strain by crystal violet binding technique when cells were grown on BHA at 24 h at 37 °C. The pYV-bearing cells are shown as small dark-violet colonies and pYV-less cells are shown as large white colonies.

presence of oxygen due to the creation of reactive oxygen species as part of the irradiation process (Thayer and Boyd 1999). Radiation sensitivity of the 3 serovars increased when CO<sub>2</sub> concentrations reached 75% and 100%. A graph of the pooled radiation D<sub>10</sub> for the 3 *Y. enterocolitica* is shown in Figure 2, as well as the predictive equation used to describe the relationship between D<sub>10</sub> and CO<sub>2</sub> concentration. The increased sensitivity of foodborne pathogens to CO<sub>2</sub> has also been described by other researchers. Grant and Patterson (1991) noted the effect of irradiation and CO<sub>2</sub> on *Y. enterocolitica* suspended in pork. Thayer and Boyd (1999) described the increased radiation sensitivity of *L. monocytogenes* suspended in raw ground beef in high CO<sub>2</sub> environments.

Exposure to ultraviolet radiation, antibiotics that inhibit the enzyme DNA gyrase, or exposure to genotoxic chemicals induce the loss of plasmids in bacteria and fungi (Danilevskaya and Gregarov 1980; Cejka and others 1982; Cansado and others 1989; Nakamura 1990; Sommers and Bhaduri 2001). Many foodborne pathogens carry plasmids that contribute to their virulence, including Shiga-toxin producing *Escherichia coli* (STEC) *Salmonella* spp., and *Campylobacter* (Butler and others 1987; Johnson and Nolan 2009; Farshad and others 2012; Feng and others 2012). Virulence of *Yersinia* spp. is dependent on the presence of a family of structurally related plasmids (Cornelis and others 1998). Loss of pYV or mutation of pYV-encoded virulence genes, leads to attenuation of *Y. enterocolitica* strains (Darwin and Miller 1999).

In addition to damaging genetic material, gamma radiation also disrupts cell membrane associated DNA complexes required for plasmid partitioning and active sites for the DNA repair process (Watkins 1980; Khare and others 1982; Naidu and others 1998). Gamma radiation induces DNA strand-breaks, transition mutations, transversion mutations, frameshift mutations, and deletions (Glickman and others 1980; Raha and Hutchison 1991; Sargentini and Smith 1994; Wijker and others 1996; Novak and others 2005). Wijker and others (1996) recommended a gamma radiation dose of 0.25 kGy, which decreased survival to 2%, for proper selection and characterization of mutants in *E. coli* strain EC919. Gamma radiation doses that induce 5 log reductions used in food processing severely damage bacterial chromosomal DNA (Sommers and Novak 2002; Novak and others 2005). Because plasmids are epigenetic elements, and normally not essential for survival, they can be lost without impairing cell viability. Kim and Thayer

(1995) verified that strand breakage caused by gamma radiation significantly damaged plasmid DNA in *Salmonella*.

Unlike foodborne pathogens such as *Salmonella* spp. or STEC, it is possible to screen large numbers of *Y. enterocolitica* for the presence or absence of pYV through the use of the CV binding assay in which colonies that contain pYV bind CV and are relatively small when grown at 37 °C, while pYV-less isolates do not bind CV (Figure 1). In our laboratory, we have previously demonstrated that gamma radiation can induce loss of pYV in 4% to 5% of *Y. enterocolitica* suspended in vacuum-packed pork (Sommers and Bhaduri 2001). In contrast, other environmental stresses such as temperature, atmosphere, culture conditions, freezing, salt, and acidic pH have not been shown to induce pYV loss, and *Y. enterocolitica* retain the pYV and are still capable of causing food poisoning (Bhaduri 1995, 2005, 2006, 2011, 2014; Bhaduri and others 1988, 1993, 2011; Bhaduri and Phillips 2011; Bhaduri and Smith 2012, 2013; Bhaduri and Sommers 2008). Figure 3 shows the percentage of colonies which failed to bind CV in the presence of air, or combinations of CO<sub>2</sub> or N<sub>2</sub>. High CO<sub>2</sub> concentrations are thought to inactivate bacteria through lowering of intracellular pH (Daniels and others 1985). The percentage of colonies that failed to bind CV was greatest for the *Y. enterocolitica* serovars suspended in air, over that of the pathogen irradiated in the presence of CO<sub>2</sub> and/or N<sub>2</sub>, at radiation doses of 0.75 and 1.0 kGy (ANOVA,  $P < 0.05$ ). Modified atmosphere itself (combinations of CO<sub>2</sub> and/or N<sub>2</sub>) had no effect on pYV loss for *Y. enterocolitica* that survived irradiation (Figure 3). Although gamma radiation damages membranes, which could lead to increased permeability of the cell to CO<sub>2</sub> and affect viability, there was no additive effect regarding pYV loss.

When irradiated *Y. enterocolitica* was allowed to proliferate in pork, increased loss of pYV was observed, which is typically not observed in nonirradiated pork products (Bhaduri 2014; Bhaduri and Smith 2013). In this study pYV loss approached 30% when incubated for 24 h (25 °C) (Table 2). Because virulence of a microorganism is more critical than its mere presence or absence as a species in a food product, the effect of intervention technologies on virulence is crucial for accurate assessment of risk. Detection technologies for foodborne pathogens must assess not only presence, but actual virulence potential of bacteria in foods.

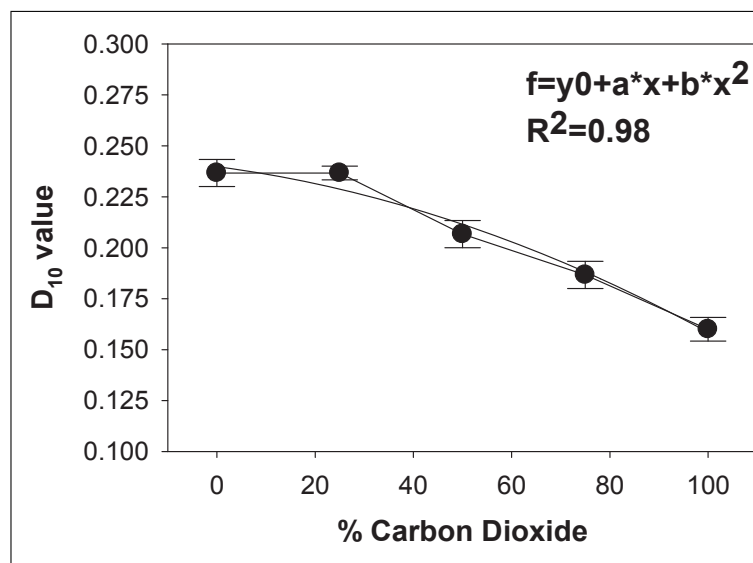
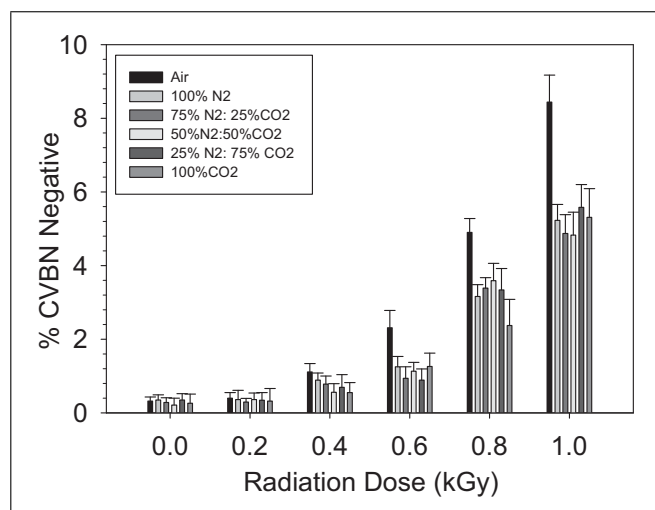


Figure 2—The effect of ionizing radiation and carbon dioxide concentration on the radiation resistance (D<sub>10</sub>) of *Y. enterocolitica* suspended in raw ground pork. The mean gamma radiation D<sub>10</sub> values for GER, 51871, and PT18-1 suspended in raw ground pork in the presence of nitrogen and carbon dioxide. Standard deviation is shown by error bars. D<sub>10</sub> values were statistically lower at 50%, 75%, and 100% CO<sub>2</sub> versus the 0% CO<sub>2</sub>: 100% N<sub>2</sub> control as determined by ANOVA ( $P < 0.05$ ).





**Figure 3—The effect of ionizing (gamma) radiation and carbon dioxide on crystal violet binding of *Y. enterocolitica* suspended in raw ground pork. The mean percentage of crystal violet binding loss (CVBN) for large virulence plasmid containing GER, 51871, and PT18–1 suspended in raw ground pork following gamma irradiation in the presence of air versus nitrogen and carbon dioxide MAP. The standard error of the mean is shown by error bars. The CVBN% was significantly greater in air as opposed to modified atmospheres containing N<sub>2</sub> and/or CO<sub>2</sub> at 0.6, 0.8 and 1.0 kGy (ANOVA,  $P < 0.05$ ).**

**Table 2—Crystal violet binding loss after 24-h pathogen growth at 25 °C following modified atmosphere packaging and gamma irradiation.**

Atmosphere	% CVB Loss (SEM)
Air	36.4 (5.50)
100% N <sub>2</sub>	34.5 (3.25)
75% N <sub>2</sub> : 25% CO <sub>2</sub>	32.1 (4.93)
50% N <sub>2</sub> : 50% CO <sub>2</sub>	30.6 (3.00)
25% N <sub>2</sub> : 75% CO <sub>2</sub>	35.6 (6.71)
100% CO <sub>2</sub>	32.0 (3.56)

Mean crystal violet binding loss for the 3 *Y. enterocolitica* serovars. Standard error of the mean is shown in parenthesis. There was no statistical difference in crystal violet binding loss between atmospheres as determined by ANOVA ( $P = 0.089$ ).

## Conclusions

High CO<sub>2</sub> concentration as part of MAP increases the radiation sensitivity of *Y. enterocolitica* suspended in raw ground pork. Gamma radiation, but not CO<sub>2</sub> concentration, affected pYV loss. The pYV loss was greatest when irradiation took place in the presence of air (oxygen). Most importantly, this study highlights the importance of actual virulence of foodborne pathogens, as opposed to mere presence, when assessing risk of actual foodborne illness.

## Acknowledgments

We would like to thank Laren Melenski and Kimberly Brobst-Striny for their technical assistance.

## References

Atkinson S, Goldstone R, Joshua P, Chang C-Y, Patrick, Camara M, Wren B, Williams P. 2011. Biofilm development on *Caenorhabditis elegans* by *Yersinia* is facilitated by quorum sensing-dependent repression of type III secretion. *PLoS Pathog* 7:1–12.

Bhaduri S. 1995. Effect of enrichment procedures on the stability of virulence plasmid and virulence-associated characteristics in *Yersinia enterocolitica*. *J Rapid Methods Autom Microbiol* 3:215–22.

Bhaduri S. 2003. A comparison of sample preparation methods for PCR detection of pathogenic *Yersinia enterocolitica* from ground pork using swabbing and slurry homogenate techniques in a single enrichment medium. *Mol Cell Probes* 17:99–105.

Bhaduri S. 2005. Survival, injury, and virulence of freeze-stressed plasmid-bearing virulent *Yersinia enterocolitica* in ground pork. *Foodb Pathog Dis* 2:353–6.

Bhaduri S. 2006. Enrichment, isolation, and virulence of freeze-stressed plasmid-bearing virulent *Yersinia enterocolitica* in pork chops. *J Food Prot* 69:1983–5.

Bhaduri S. 2011. Effect of salt and acidic pH on the stability of virulence plasmid (pYV) in *Yersinia enterocolitica* and expression of virulence associated characteristics. *Food Microbiol* 28:171–3.

Bhaduri S. 2014. Food-borne pathogenic *Yersinia enterocolitica*. In: R Robinson, C Batt, ML Tortorello, editors. *Encyclopaedia of food microbiology*. London, UK: Academic Press Ltd. in press.

Bhaduri S, Chaney K, Smith JL. 2011. A procedure for monitoring the presence of the virulence plasmid (pYV) in *Yersinia pestis* under culture conditions. *Foodb Pathog Dis* 8:459–63.

Bhaduri S, Phillips J. 2011. Growth model of a plasmid-bearing virulent strain of *Yersinia pseudotuberculosis* in raw ground beef. *Zoonoses Public Health* 58:77–84.

Bhaduri S, Smith JL. 2011. Stress responses in pathogenic *Yersinia enterocolitica* with reference to the stability of the virulence plasmid in food. In: H-C Wong, editor. *Stress responses of foodborne microorganisms*. Hauppauge, N.Y.: Nova Science Publishers, Inc.

Bhaduri S, Smith JL. 2012. Virulence plasmid (pYV)-associated expression of phenotypic virulent determinants in pathogenic *Yersinia* species: a convenient method for monitoring the presence of pYV under culture conditions and its application for isolation/detection of *Yersinia pestis* in food. *J Pathog doi:10.4061/2011/727313*.

Bhaduri S, Smith JL. 2013. Pathogenic *Yersinia enterocolitica*. In: RH Labbe, JS Garcia-Alvarado, editors. *Guide to foodborne pathogens*. New York, N.Y.: John Wiley and Sons, Inc. p 177–187.

Bhaduri S, Sommers C. 2008. Detection of *Yersinia pestis* by comparison of virulence plasmid (PYV/PCD)-associated phenotypes in *Yersinia* species. *J Food Safety* 28:453–66.

Bhaduri S, Turner-Jones C. 1993. The effect of anaerobic atmospheres on the stability of the virulence-related characteristics in *Yersinia enterocolitica*. *Food Microbiol* 10:239–42.

Bhaduri S, Turner-Jones C, Conway LK. 1988. Stability of virulence plasmid in *Yersinia enterocolitica* at elevated temperatures. *Food Microbiol* 5:231–3.

Butler R, Lund V, Carlson D. 1987. Susceptibility of *Campylobacter jejuni* and *Yersinia enterocolitica* to UV radiation. *Appl Environ Biol* 53:375–8.

Cansado J, Longo E, Agrelo D, Villa T. 1989. Curing of the killer character of *Saccharomyces cerevisiae* with acridine orange. *FEMS Microbiol Lett* 53:233–7.

Cejka K, Holubova I, Hubacek J. 1982. Curing effect of cloribocin on *Escherichia coli* plasmids. *Mol Gen Genet* 186:153–5.

Cornelis G, Boland A, Boyd A, Geuijin C, Iriarte M, Neyt C, Sory C, Stanier I. 1998. The virulence plasmid of *Yersinia*, an antihist genome. *Microbiol Mol Biol Res* 62:1315–52.

Daniels J, Krishnamurthy R, Rizvi S. 1985. A Review of Effects of CO<sub>2</sub> on microbial growth and Food Quality. *J Food Prot.* 48:532–7.

Danilevskaia O, Gregorov A. 1980. Curing of *Escherichia coli* K12 plasmids by coumermycin. *Mol Gen Genet.* 178:233–5.

Darwin A, Miller L. 1999. Identification of *Yersinia enterocolitica* genes affecting survival in an animal host using signature-tagged transposon mutagenesis. *Mol Microbiol* 32:51–62.

Diehl J. 1995. Safety of irradiated food. 2nd ed. New York, N.Y.: Marcel Dekker. p 93–8.

United States Food and Drug Administration (FDA). 1997. Irradiation in the production, processing, and handling of food. *Fed Reg.* 231(64):64107–21.

Farshad S, Ranjbar R, Japoni A, Hosseini M, Anvarinejad M, Mohammadzadegan R. 2012. Microbial susceptibility, virulence factors, and plasmid profiles of uropathogenic *Escherichia coli* strains isolated from children in Jahrom. *Iran Arch Iran Med* 15:312–6.

Feng Y, Li Y-G, Cao F-L, Johnston R, Zhou J, Liu G-R, Liu S-L. 2012. Inheritance of the *Salmonella* virulence plasmids: mostly vertical and rarely horizontal. *Infect Genet Evol* 12(5):1058–63.

Freund S, Czech B, Trulzsch K, Ackermann N, Heesemann J. 2008. Unusual virulence plasmid-dependent growth behavior of *Yersinia enterocolitica* in three-dimensional collagen gels. *J Bacteriol* 190:4111–20.

Glickman B, Rietveld K, Aaron C. 1980.  $\gamma$ -Ray induced mutational spectrum in the *lacI* gene of *Escherichia coli*. *Mutat Res* 69:1–12.

Grant I, Patterson M. 1991. Effect of irradiation and modified atmosphere packaging on the microbiological and sensory quality of pork stored at refrigeration temperatures. *Int J Food Sci Technol* 26:507–19.

Johnson T, Nolan K. 2009. Pathogenomics of virulence plasmids of *Escherichia coli*. *Microbiol Mol Biol Rev* 73:750–74.

Kamat A, Khare S, Doctor T, Nair P. 1997. Control of *Yersinia enterocolitica* in raw pork and pork products by  $\gamma$  irradiation. *Int J Food Microbiol* 36:69–76.

Kato T, Oda Y, Glickman B. 1985. Randomness of base substitution mutations induced in the *lacI* gene of *Escherichia coli* by ionizing radiation. *Rad Res* 101:402–6.

Khare S, Jayakumar A, Trevvedi A, Kasevan P, Presad R. 1982. Radiation effects on membranes. *Rad Res* 90:233–43.

Kim A, Thayer D. 1995. Radiation-induced cell lethality of *Salmonella typhimurium* ATCC 14028: cooperative effect of hydroxyl radical and oxygen. *Rad Res* 144:36–42.

Kudra L, Sebranek JG, Dickson JS, Mendonca A, Larson E, Jackson-Davis A, Lu Z. 2011. Effects of vacuum or modified atmosphere packaging in combination with irradiation for control of *Escherichia coli* O157:H7 in ground beef patties. *J Food Prot* 74:2018–23.

Kim A, Thayer D. 1996. Mechanism by which gamma irradiation increases the heat sensitivity of *Salmonella typhimurium* ATCC 14028 to heat. *Appl Environ Microbiol* 62:1759–63.

Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV. 1999. Food-related illness and death in the United States. *Emerg Infect Dis* 5:607–25.

Naidu M, Chander R, Nair P. 1998. Effect of gamma irradiation on chemical and biological properties of lipopolysaccharide from *Salmonella typhimurium*. *Indian J Exp Biol* 36:588–92.

Nakamura S. 1990. Curing effects of chlorination, ozone, and UV treatments on plasmid DNA's. *Nippon Koshu Eisei Zasshi* 37:745–51.

Novak J, Sommers C, Juneja V. 2005. A convenient method to detect potentially lethal heat-induced damage to DNA in *Clostridium perfringens*. *Food Ctr* 16:399–404.

Raha M, Hutchison F. 1991. Deletions induced by gamma rays in the genome of *Escherichia coli*. *J Mol Biol* 220:193–8.

Sargentini N, Smith, K. 1994. DNA sequence analysis of  $\gamma$ -radiation (anoxic)-induced and spontaneous *lacI* mutations in *Escherichia coli* K-12. *Mutat Res* 309:147–63.

Scallan E, Hoekstra R, Angulo F, Tauxe R, Widdowson M, Roy S, Jones J, Griffin P. 2011. Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis* 17:7–12.

- Sommers C, Bhaduri S. 2001. Loss of crystal violet binding activity in stationary phase *Yersinia enterocolitica* following gamma irradiation. Food Microbiol 18:367–74.
- Sommers C, Niemira B, Tunick M, Boyd G. 2002. Effect of temperature on the radiation resistance of virulent *Yersinia enterocolitica*. Meat Sci 61:323–8.
- Sommers C, Novak J. 2002. Radiation resistance of virulence plasmid-containing and plasmid-less *Yersinia enterocolitica*. J Food Prot 65: 556–9.
- Thayer D, Boyd G. 1999. Irradiation and modified atmosphere packaging for the control of *Listeria monocytogenes* on turkey meat. J Food Prot 62:1136–42.
- Watkins D. 1980. Stimulation of DNA synthesis in bacterial DNA-membrane complexes after low doses of ionizing radiation. Int J Radiat Biol Relat Chem Med 38:247–56.
- Wijker C, Lafleur M, van Steeg H, Mohn G, Retel J. 1996.  $\gamma$ -Radiation-induced mutation spectrum in the episomal lacI gene of *Escherichia coli* under oxic conditions. Mutat Res 349:229–39.
-